#041

Claims

1. A method for producing a zinc finger nucleic acid binding protein comprising preparing a zinc finger protein according design rules, varying the protein at one or more positions, and selecting variants which bind to a target nucleic acid sequence by polysome display.

2. A method according to claim 1, wherein the protein is varied at one or more positions selected from the group consisting of +1, +5, +8, -1, +2, +3 and +6.

3. A method for producing a zinc finger nucleic acid binding protein comprising an at least partially varied sequence and selecting variants thereof which bind to a target DNA strand, comprising the steps of:

(i) preparing a nucleic acid binding protein of the Cys2-His2 zinc finger class capable of binding to a nucleic acid triplet in a target nucleic acid sequence, wherein binding to each base of the triplet by an α-helical zinc finger nucleic acid binding motif in the protein is determined as follows:

a) if the 5' base in the triplet is G, then position +6 in the α-helix is Arg; or position +6 is Ser or Thr and position ++2 is Asp;

b) if the 5' base in the triplet is A, then position +6 in the α-helix is Gln and ++2 is not Asp;

c) if the 5' base in the triplet is T, then position +6 in the α-helix is Ser or Thr and position ++2 is Asp;

d) if the 5' base in the triplet is C, then position +6 in the α-helix may be any amino acid, provided that position ++2 in the α-helix is not Asp;

e) if the central base in the triplet is G, then position +3 in the α-helix is His;

f) if the central base in the triplet is A, then position +3 in the α-helix is Asn;

g) if the central base in the triplet is T, then position +3 in the α-helix is Ala, Ser or Val; provided that if it is Ala, then one of the residues at -1 or +6 is a small residue;

h) if the central base in the triplet is C, then position +3 in the α-helix is Ser, Asp, Glu, Leu, Thr or Val;

i) if the 3' base in the triplet is G, then position -1 in the α-helix is Arg;

j) if the 3' base in the triplet is A, then position -1 in the α-helix is Gln;

k) if the 3' base in the triplet is T, then position -1 in the α-helix is Asn or Gln;

l) if the 3' base in the triplet is C, then position -1 in the α-helix is Asp;

(ii) varying the resultant polypeptide at at least one position; and

(iii) selecting the variants which bind to a target nucleic acid sequence by polysome display.

4. A method according to any preceding claim, wherein the or each zinc finger has the general primary structure

(A) X^a **C** X_{2-4} **C** X_{2-3} F X^c X X X X L X X **H** X X X^b **H** - linker

-1 1 2 3 4 5 6 7 8 9

wherein X (including X^a, X^b and X^c) is any amino acid.

5. A method according to claim 5 wherein X^a is $^F/_Y$-X or P-$^F/_Y$-X.

6. A method according to claim 4 or claim 5 wherein X_{2-4} is selected from any one of: S-X, E-X, K-X, T-X, P-X and R-X.

7. A method according to any one of claims 4 to 6 wherein X^b is T or I.

8. A method according to any one of claims 4 to 7 wherein X_{2-3} is G-K-A, G-K-C, G-K-S, G-K-G, M-R-N or M-R.

9. A method according to any one of claims 4 to 8 wherein the linker is T-G-E-K or T-G-E-K-P.

10. A method according to any one of claims 4 to 9 wherein position +9 is R or K.

11. A method according to any one of claims 4 to 10 wherein positions +1, +5 and +8 are not occupied by any one of the hydrophobic amino acids, F, W or Y.

12. A method according to claim 11 wherein positions +1, +5 and +8 are occupied by the residues K, T and Q respectively.

13. A method according to any preceding claim, wherein the polysome display technique comprises the steps of:

(a) introducing a population of mRNA species into an in vitro translation system under conditions suitable for translation to form a pool of polysomes displaying nascent zinc finger polypeptides;

(b) contacting the polysomes with a target nucleic acid under suitable binding conditions;

(c) selecting polysomes which are specifically bound to the nucleic acid; and

(d) reverse transcribing and amplifying the isolated mRNA.